

California All Taxa Biodiversity Inventory Sampling Protocol: Environmental DNA

The following instructions are for environmental DNA (eDNA) sample collection. Do not collect eDNA samples on or near archeological sites. Archeological sites include areas where artifacts or cultural material are located as well as in or around historical structures. If you do encounter an archeological site while sampling, do not move or remove archeological material, stop sampling, and move locations.

If you are collecting soil samples, you do not need to collect eDNA samples. During processing, soil samples will be used for eDNA analysis.

Precautions and Guidance on Sampling:

- **Use caution to prevent contaminating your sample with DNA that is not from the site.**
- Be sure to clean the spade prior to sampling with a bleach wipe or 10% bleach solution. Allow the bleach to evaporate off the spade face prior to sampling (~30 seconds).
- Try not to touch the soil sample if possible. Use a stick or rock from the site instead of your finger to pack soil down.
- If you do have to touch the sample, make sure you are wearing clean gloves that have not been used to touch your backpack, face, clothing etc.
- If you do touch or contaminate the sample, then discard and start over.
- Avoid rocks, sticks, mushrooms, leaves or other large debris or organic material.
- Collect samples in natural areas that are not impacted by anthropogenic disturbances. If possible, sample away from roads, trails, etc.
- **DO NOT sample in agriculture/ agricultural fields.**

Equipment:

- Gloves
- Barcode sticker labels or pre-labeled CRYOtube (1 per site)
- 5- milliliter (mL) CRYOtube (1 per site)
- Whirl-Pak or Ziplock bag for storing CRYOtubes (1 per site)
- Spade (sticks or rocks could be used)
- 15-cm ruler (optional)
- Bleach wipes or 10% bleach solution for sterilization

Steps:

1. Put on a pair of clean gloves.
2. If using the spade, wipe it with a bleach wipe and let dry (30 seconds).
3. Using the spade or natural material (rock or stick) found at the site, scrape away debris and litter from the collection area.
4. Using the spade or natural material (rock or stick) found at the site, collect approximately 1.5 mL of soil and put it in the CRYOtubes. Soil should be collected at approximately 1cm depth. Pack soil down in CRYOtube using a stick found at the site to avoid touching the sample if possible.
5. Repeat steps 3. and 4. at two other locations within 1 foot of each other to collect a total of 4.5 mL soil sample.
6. Place lid on CRYOtube.
7. Scan the barcode label.
8. Store CRYOtubes in a cool, dark, and dry location (e.g. in a cooler with leakproof ice packs or in a fridge). If a cooler or fridge is unavailable, store CRYOtubes in an insulated bag out of direct sunlight. Do NOT freeze samples.